

Stochastic Models of Soil Denitrification

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Received 18 July 1988/Accepted 19 October 1988

Soil denitrification is a highly variable process that appears to be lognormally distributed. This variability is manifested by large sample coefficients of variation for replicate estimates of soil core denitrification rates. Deterministic models for soil denitrification have been proposed in the past, but none of these models predicts the approximate lognormality exhibited by natural denitrification rate estimates. In this study, probabilistic (stochastic) models were developed to understand how positively skewed distributions for field denitrification rate estimates result from the combined influences of variables known to affect denitrification. Three stochastic models were developed to describe the distribution of measured soil core denitrification rates. The driving variables used for all the models were denitrification enzyme activity and CO₂ production rates. The three models were distinguished by the functional relationships combining these driving variables. The functional relationships used were (i) a second-order model (model 1), (ii) a second-order model with a threshold (model 2), and (iii) a second-order saturation model (model 3). The parameters of the models were estimated by using 12 separate data sets (24 replicates per set), and their abilities to predict denitrification rate distributions were evaluated by using three additional independent data sets of 180 replicates each. Model 2 was the best because it produced distributions of denitrification rate which were not significantly different ($P > 0.1$) from distributions of measured denitrification rates. The generality of this model is unknown, but it accurately predicted the mean denitrification rates and accounted for the stochastic nature of this variable at the site studied. The approach used in this study may be applicable to other areas of ecological research in which accounting for the high spatial variability of microbiological processes is of interest.

It is generally recognized that soil denitrification is influenced primarily by three factors: (i) soil aeration status (moisture), (ii) available organic C, and (iii) NO₃⁻ concentration (6). These and other factors have been combined in various mathematical relationships either to predict field denitrification rates (4, 10, 14, 16, 28) or to investigate their influence on observed denitrification variability (2, 3, 17, 26). All these models are deterministic in nature, yet natural rate estimates of soil denitrification typically exhibit high spatial variability (8, 17, 23, 25). It may be unreasonable to expect that a highly variable process can be adequately described by a deterministic model. Indeed, deterministic models do not account for the probabilistic and highly variable nature of soil denitrification.

In a study of soil aeration, Flüher et al. (7) found that variability of soil aeration could be described as a statistical expression of microscale variation and concluded that frequency distributions of aeration measurements may describe soil aeration status better than mean values. Since, in well-drained soils, microsite aeration status significantly influences denitrification (29, 30) a probabilistic approach to modeling denitrification may be of value.

The objective of the work described here was to determine whether the frequency distributions of CO₂ production rate and denitrification enzyme activity can be combined in mathematical relationships to accurately predict the variation exhibited by natural denitrification rate estimates. This approach is termed stochastic modeling (9, 12), since the model output is a distribution of values rather than a single prediction.

MATERIALS AND METHODS

Study site and sample collection. The study site was located in the Eastern Shore region of Maryland at the Wye Research and Education Center near Queenstown, Md. The soil was a well-drained Matapeake silt loam (fine-silty, mixed, mesic, Typic Hapludults) having a pH of 6.5 and an organic C content of 7.2 mg of C/g. Three replicate field plots (4.6 by 12.2 m) had been cropped to continuous corn for 4 years by no-tillage practices with an N fertilization rate of 180 kg of N/ha (surface broadcast NH₄NO₃ in the spring). Soil cores were obtained by pounding a steel coring tube (4-cm inner diameter) containing a plastic cylinder insert into the ground to a depth of 16 cm. The plastic insert containing the intact soil core was then removed from the coring tube and stoppered at both ends. The plots were sampled on 12 dates in the spring and fall of 1986 (eight cores per plot), and data from these samples were used to estimate the parameters of the nonlinear models. On three subsequent dates, additional samples were collected (180 cores per sample date) from a single plot, and data from these samples were used to validate the models.

Denitrification and CO₂ production rate measurements. Denitrification and CO₂ production rate measurements were begun immediately upon returning to the laboratory. Natural denitrification rates of the intact soil cores were estimated by a C₂H₂ block technique. First, the gas pressure in each core was brought to atmospheric levels by venting the cores with a needle. After the cores were vented, 10 ml of C₂H₂ was added to each core, resulting in a C₂H₂ partial pressure of ca. 10 kPa. The pressure increase resulting from the C₂H₂ additions was then observed in each core with a pressure transducer equipped with a 55.2-kPa (8-lb/in²) bellows (Unimeasure Inc., Grants Pass, Oreg.). The pressure readings were used to calculate the total gas-filled volume of each

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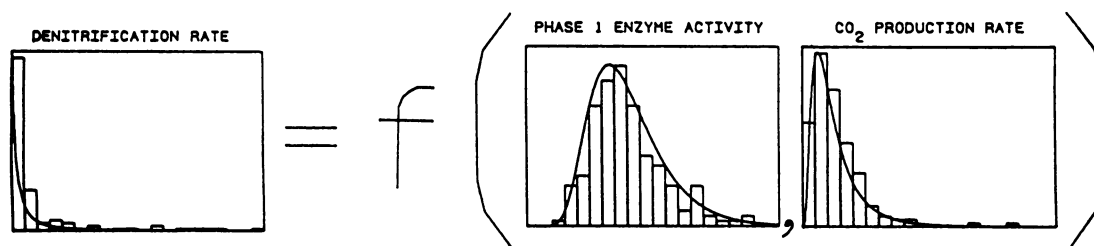


FIG. 1. General form of stochastic models.

sample as described by Parkin et al. (21). The C_2H_2 used for the denitrification incubations was generated by reacting CaC_2 with distilled water immediately prior to use.

Gas in the soil cores was mixed to distribute C_2H_2 throughout the soil pores. Mixing was accomplished by alternately drawing and releasing a vacuum on the samples by using a 60-ml syringe. The mixing procedure and the loose fit of the intact soil cores in the tubes facilitated both C_2H_2 distribution into and N_2O distribution out of the soil pores.

Following the gas mixing, the overpressure of gas in the soil cores resulting from the initial C_2H_2 injection was vented. Cores were incubated at 24 to 26°C, and gas samples were withdrawn at 3, 6, and 18 h following the C_2H_2 mixing. Gas samples were obtained by adding 5 ml of air to each core, mixing the gas in the cores by the above procedure, and then removing a 5-ml gas sample. The 5-ml gas samples were stored in 3-ml evacuated vials for later N_2O and CO_2 analyses.

Contents of the vials were analyzed for N_2O with an electron capture detector-gas chromatograph, and CO_2 was determined with a gas chromatograph equipped with a thermal conductivity detector. Both gas chromatographs were fit with automatic gas-sampling devices (19). The water contents of the samples were determined gravimetrically after air drying, and rates of N_2O and CO_2 production are expressed on a dry weight basis.

Denitrification enzyme activity. Denitrification enzyme activity (phase 1 rate) was determined immediately following the last gas sampling of the intact core incubations (31, 32). The soil samples were sieved and mixed, and a 25-g subsample was placed in a 125-ml Erlenmeyer flask containing 25 ml of a glucose-nitrate-chloramphenicol solution (1 mM glucose, 1 mM KNO_3 , 1 g of chloramphenicol per liter). The soil slurries were made anaerobic by alternately flushing with Ar and evacuating the flasks four times. Gas samples of 5 ml were withdrawn 0.5, 1, 1.5, and 2 h following the addition of 20 ml of C_2H_2 to the flasks. Gas samples were stored in evacuated vials and analyzed for N_2O as described above.

Modeling and statistical analyses. Estimates of the mean, variance, and coefficient of variation for the lognormally distributed variables were calculated by the unbiased minimum variance method of Finney (5) as described by Parkin et al. (22). The mean and variance estimates defined the two-parameter lognormal probability density functions used in the Monte Carlo simulations during model implementation. Parameter estimates for the models were obtained by fitting the 12 data sets (mean denitrification rate versus mean CO_2 rate and mean phase 1 rate) to the three nonlinear models by the technique of nonlinear least squares (SAS PROC NLIN).

The models were evaluated by comparing predicted means and variances with the measured means and variances from

three validation data sets. Means were compared by observing the overlap of the 95% confidence limits, which were calculated by the procedure of Land (13). Variances were compared by a permutation test. For this analysis, variance estimates were obtained from 1,000 runs of each model and the difference between the measured variance and the predicted variance was calculated. Predicted variances were judged to be not significantly different from the measured variance if the 90% confidence interval of the population of differences included zero.

With the exception of the nonlinear regression analyses, all statistical analyses and modeling (including the Monte Carlo simulations) were done with a microcomputer (MS/DOS). Programs were written in BASIC and are available upon request.

RESULTS

Model building and parameter estimation. It was hypothesized that the highly skewed frequency distributions exhibited by natural rate estimates of soil denitrification were functionally related to the frequency distributions of potential denitrification enzyme activity and CO_2 production in soil (Fig. 1). The phase 1 measurements of Smith and Tiedje (31) indicate the maximum potential activity of existing denitrifying enzymes in soil (32). Thus, in our models, phase 1 enzyme activity is viewed as proportional to the maximum rate of denitrification which could be expected under optimal denitrifying conditions (V_{max}). We used CO_2 production as a driving variable for the expression of denitrification enzyme activity in our models, under the assumption that CO_2 production activity indicates both organic C availability as well as soil aeration status (i.e., O_2 consumption activity).

Three different functions relating natural denitrification rate to CO_2 production and phase 1 enzyme activity were proposed and evaluated: (i) a second-order model (equation 1), (ii) a second-order model with a threshold term for CO_2 production rate (equation 2), and (iii) a second-order saturation model (equation 3). These are designated as models 1, 2, and 3, respectively.

(i) *Model 1.*

$$\text{Denitrification} = K_1 \times \text{phase 1} \times CO_2 \quad (1)$$

where K_1 is the pseudo second-order rate coefficient.

(ii) *Model 2.*

$$\begin{aligned} \text{Denitrification} &= K_1 \times \text{phase 1} \times (CO_2 - K_2); CO_2 > K_2 \\ \text{Denitrification} &= 0.05 \text{ ng of N/g per day}; CO_2 \leq K_2 \end{aligned} \quad (2)$$

where K_1 is the pseudo second-order rate coefficient, K_2 is the threshold rate of CO_2 production, and 0.05 is the detection limit of the denitrification rate assay.

(iii) *Model 3.*

$$\text{Denitrification} = (K_1 \times \text{phase 1} \times CO_2) / (K_2 + CO_2) \quad (3)$$

TABLE 1. Mean rates of denitrification, phase 1 enzyme activity, and CO₂ production used to generate model parameters

Sampling date	Denitrification rate (ng of N/g per day)	Phase 1 rate (μg of N/g per day)	CO ₂ rate (μg of C/g per day)
6/13/86	7.32	3.33	4.16
6/20/86	1.66	3.44	2.35
6/24/86	3.01	4.68	2.49
6/25/86	0.32	2.58	2.40
7/03/86	0.45	4.27	1.64
7/14/86	0.22	3.68	1.42
7/16/86	0.32	2.86	0.94
7/21/86	1.73	6.68	3.12
8/04/86	0.53	3.08	2.50
10/14/86	6.49	2.67	2.29
10/16/86	6.62	3.65	5.04
10/22/86	0.41	2.61	2.71

where K_1 is the pseudo second-order rate coefficient and K_2 is the CO₂ production rate at which the denitrification rate is half of its maximum rate at a saturating level of substrate concentration.

Parameter estimates were obtained for each model by regressing the mean denitrification rate versus the mean rates of phase 1 enzyme activity and CO₂ production. Twelve data sets (24 intact soil cores per set) collected from no-till plots in the summer and fall of 1986 (Table 1) were used in the regression analyses to estimate the parameter values for each model. For model 1, an estimate of $K_1 = 0.252$ was obtained. For model 2, a value of 0.450 was obtained for K_1 and K_2 (the CO₂ threshold) had a value of 1.36. For model 3, unbiased parameter estimates could not be obtained, indicating that our data were too noisy to obtain unique parameter estimates for this two-parameter model.

Coefficients of determination (r^2) for models 1 and 2 were low (0.244 and 0.338, respectively) but significant at the 0.05 probability level. These coefficients only indicate goodness of fit associated with predicted mean denitrification rates and not the variances associated with these means or the distributions of the predicted denitrification rate estimates.

Model implementation and evaluation. Models 1 and 2 were evaluated by using three additional data sets which were collected in the fall of 1986 and the spring of 1987. These data sets contained 180 intact soil cores each. The large number of samples composing these validation data sets allowed for an accurate determination of the frequency distributions of denitrification, phase 1 enzyme activity, and CO₂ production rate. The sample histograms of the phase 1 enzyme activity and CO₂ production rate for each of the validation data sets are presented in Fig. 2. Shown in the insets of each panel are the summary statistics for each data set. In each case, the distributions of these variables could be described by a lognormal probability density function.

In the implementation of the stochastic models, Monte Carlo simulation (9) was used to randomly select variates from the lognormal probability density functions describing the frequency distributions of phase 1 activity and CO₂ production (Fig. 2). These values were then used as driving variables in the functional relationships defined by models 1 and 2 (equations 1 and 2) to obtain a single predicted denitrification rate. This process was repeated n times ($n = 180$) to obtain a distribution of predicted rates for each of the two models. The models were then evaluated by comparing the predicted denitrification rate distributions with the measured denitrification rate distributions obtained for each validation data set.

Sample histograms along with summary statistics for measured denitrification rates and predicted denitrification rates from models 1 and 2 are shown in Fig. 3. It is evident from the shape of the histograms that model 2 gave populations of denitrification rates similar to the measured rates. Mean rate estimates obtained from model 2 were not significantly different ($P > 0.1$) from the measured rates for all three data sets, as indicated by the overlap of the 95% confidence limits. In addition, model 2 accurately described the variability exhibited by natural denitrification rate estimates. Coefficients of variation of predicted rates were very similar to the actual rates, and variances of the predicted rates from model 2 were not significantly different ($P > 0.1$) from the measured denitrification rates. Model 1 only yielded an accurate prediction of mean denitrification for validation data set 1, and variances predicted from model 1 underestimated the measured variances for all three validation data sets.

DISCUSSION

Soil denitrification typically exhibits high spatial variability, with coefficients of variation exceeding 100%. This high variability is manifested in a given data set when most samples have low (or undetectable) rates, but a few samples display very high rates. In contrast, variability of active denitrifying enzymes is comparatively low, with coefficients of variation in the range of 30 to 40% (23). Also, enzyme activity is typically measurable in every sample, indicating that the spatial dispersion of denitrifying bacteria in soil is relatively uniform. Therefore, the variability associated with natural denitrification rate estimates is not simply controlled by the presence of denitrifying enzymes, but rather by whether or not the enzyme activity present in a given sample is expressed.

Denitrification is an anaerobic process that requires nitrate and carbon. In fertilized agricultural soils, it is generally thought that NO₃⁻ is not limiting denitrification, even at the microsite level. Rather, the availability of organic carbon may be the primary factor controlling the expression of denitrification activity in soil (18). We used CO₂ production as a driving variable controlling the expression of denitrification enzyme activity in our models since CO₂ production activity indicates both organic C availability as well as soil aeration status (i.e., O₂ consumption activity). The use of CO₂ production as a predictor variable for denitrification is reasonable in light of recent observations of high denitrification and CO₂ production rates associated with particulate organic carbon in soil (20).

Since the law of proportionate effects predicts that multiplicative interactions between variables yield lognormal distributions (1), the models of our study were structured as multiplicative relationships of phase 1 enzyme activity and CO₂ production rates. Multiplicative effects have been proposed as the source of lognormal distributions exhibited by populations of bacteria in the rhizosphere and on leaf surfaces (11, 15). However, in a study of community structure, Ugland and Gray (33) suggested that multiplicative effects yield patchy spatial dispersions of species in nature, which is the ultimate cause for the skewed distributions observed. This patchy dispersion phenomenon may also be responsible for the high observed denitrification variability. Recently, a conceptual model was developed which indicates that the patchy spatial dispersion of denitrifying microsites associated with particulate carbon in soil results in skewed sample histograms of denitrification (20).

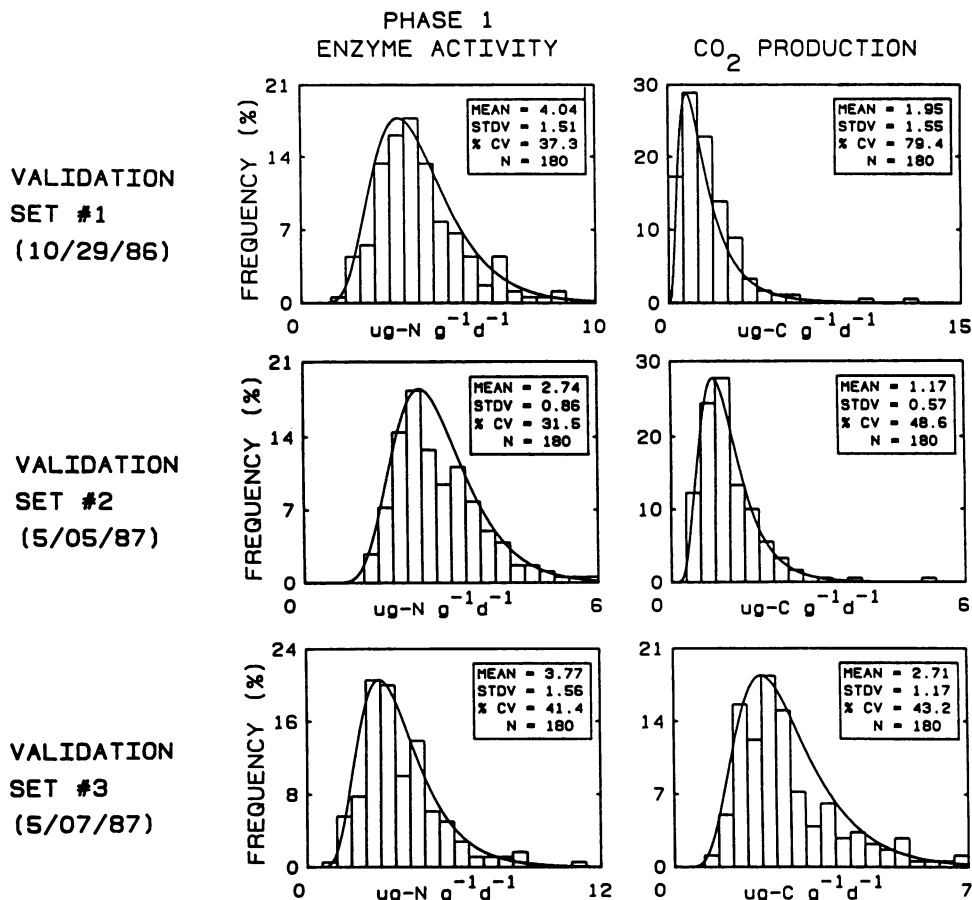


FIG. 2. Sample histograms and summary statistics for phase 1 enzyme rates and CO₂ production rates of independent data sets used in model validation. STDV, Standard deviation; % CV, percent coefficient of variation.

The threshold term in our model 2 separates the differential effects of C, which influences denitrification directly by serving as a substrate or indirectly by influencing soil oxygen status. Thus, the threshold term mathematically simulates a patchy spatial dispersion by allowing for the possibility of obtaining samples with measurable CO₂ production activity, yet having no measurable denitrification (below our detection limit of 0.05 ng of N/g per day). Model 2 codifies in mathematical terms the multiplicative influences of those factors believed to be important in the development of denitrifying hot spots in soil (20). A CO₂ threshold concept for predicting denitrification is supported by data of Rice et al. (24). In a regression model of CO₂ production versus denitrification rate, these investigators observed that a threshold CO₂ production value of 6.2 µg of C/cm² per h was necessary for "enhanced" denitrification rates. From their regression equation, we calculated a threshold CO₂ production rate required for measurable denitrification of 0.91 µg of C/g per day. This value is remarkably similar to the threshold value of 1.36 µg of C/g per day estimated for model 2 of our study.

Multiplicative interactions of enzyme activity and CO₂ production alone (model 1) could not adequately account for the extreme skewness exhibited by natural soil denitrification rate estimates. Our results also suggested that the patchy spatial dispersion and consequent mineralization of

organic carbon are the dominant factors influencing the spatial variability of soil denitrification.

Finally, the focus of our work was to develop simple probabilistic models that adequately predict mean soil core denitrification rates and account for the high variability of this process in soils. Model 2 did an effective job of predicting the mean denitrification rate, but more importantly, it accurately predicted the distribution of natural denitrification rates as a function of the distributions of CO₂ production and phase 1 rates.

Microbiologists are typically used to dealing with deterministic models (27); however, stochastic models have been used extensively in other biological sciences (9, 12). The important distinction between deterministic and stochastic models is that the latter accounts for the probabilistic nature of the variables measured in a given study. It is well known that many soil parameters are highly variable and approximately lognormally distributed. Models that fail to account for these properties, while still useful for predicting mean (average) values of the measured variables, are statistically incomplete if understanding the variability of soil processes is a desired goal. In our study, assessing factors which influence the variability of soil denitrification was a goal and thus a stochastic modeling approach was considered to be an appropriate strategy. Although we focused on soil denitrification in this study, a stochastic modeling approach may be

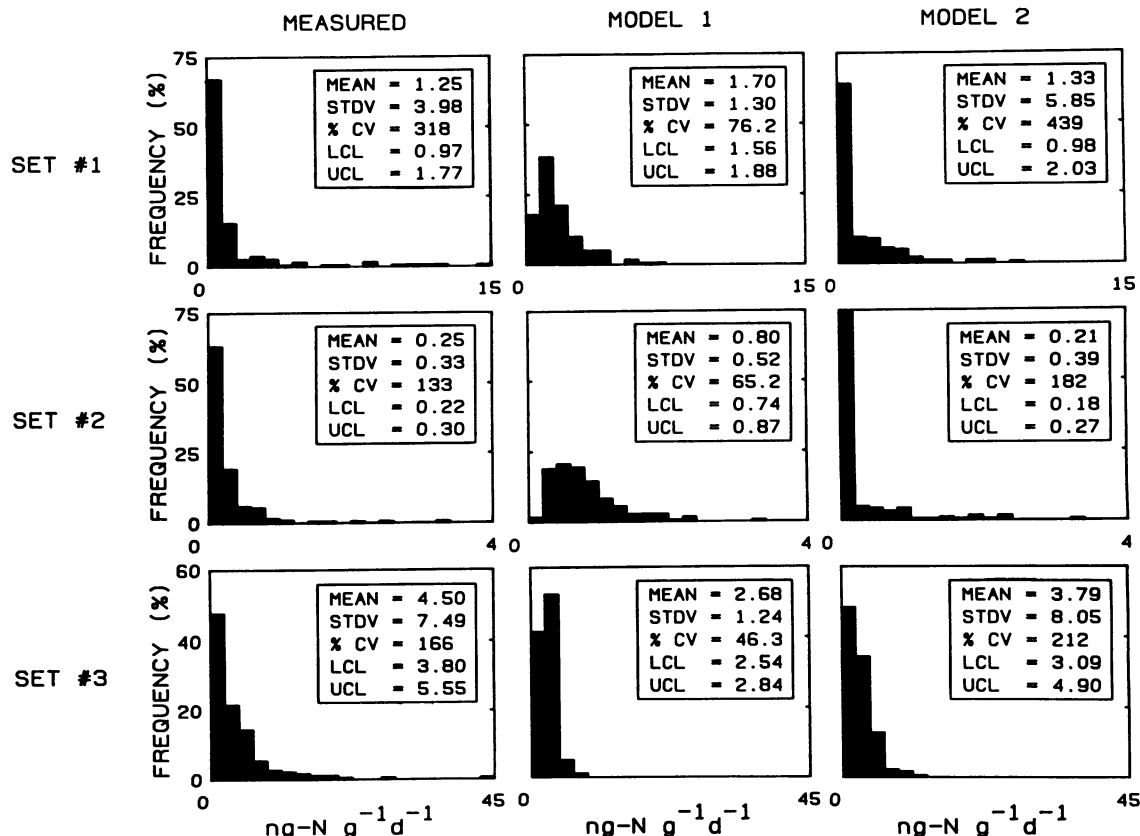


FIG. 3. Sample histograms and summary statistics for measured denitrification rates from independent validation data sets and predicted denitrification rates from models 1 and 2. STDV, Standard deviation; % CV, percent coefficient of variation; LCL, lower 95% confidence limit; UCL, upper 95% confidence limit.

useful in understanding the variability of other soil processes, such as biodegradation rates of organic chemicals.

ACKNOWLEDGMENTS

The comments, suggestions, and technical assistance of J. J. Meisinger are gratefully acknowledged. We also thank E. Codling for technical assistance in the field and laboratory and L. Smith and the University of Maryland Wye Research and Education Center for managing the field site.

LITERATURE CITED

- Aitchinson, J., and J. A. C. Brown. 1957. The lognormal distribution. Cambridge University Press, London.
- Aulakh, M. S., and D. A. Rennie. 1985. Gaseous nitrogen losses from conventional and chemical summerfallow. *Can. J. Soil Sci.* **65**:195-203.
- Burton, D. L., and E. G. Beauchamp. 1985. Denitrification rate relationships with soil parameters in the field. *Commun. Soil Sci. Plant Anal.* **16**:539-549.
- Chrisensen, S. 1985. Denitrification in a sandy loam soil as influenced by climatic and soil conditions. *Tidsskr. Planteavl* **89**:351-365.
- Finney, D. J. 1941. On the distribution of a variate whose logarithm is normally distributed. *J. R. Stat. Soc. Suppl.* **7**:144-161.
- Firestone, M. K. 1982. Biological denitrification. *Agronomy* **22**:289-326.
- Flühler, H., L. H. Stolzy, and M. S. Ardiani. 1976. A statistical approach to define soil aeration in respect to denitrification. *Soil Sci.* **122**:115-123.
- Folorunso, O. A., and D. E. Rolston. 1984. Spatial variability of field-measured denitrification gas flux. *Soil Sci. Soc. Am. J.* **48**:1214-1219.
- Grant, W. E. 1986. Systems analysis and simulation in wildlife and fisheries science. John Wiley & Sons, Inc., New York.
- Grundmann, G. L., and D. E. Rolston. 1987. A water function approximation to degree of anaerobiosis associated with denitrification. *Soil Sci.* **144**:437-441.
- Hirano, S. S., E. V. Nordheim, D. C. Arny, and C. D. Upper. 1982. Lognormal distribution of epiphytic bacterial populations on leaf surfaces. *Appl. Environ. Microbiol.* **44**:695-700.
- Ingels, D. M. 1985. What every engineer should know about computer modeling and simulation. Marcel Dekker, Inc., New York.
- Land, C. E. 1973. Standard confidence limits for linear functions of the normal mean and variance. *J. Am. Stat. Assoc.* **68**:960-963.
- Linn, D. M., and J. W. Doran. 1984. Aerobic and anaerobic microbial populations in no-till and plowed soils. *Soil Sci. Soc. Am. J.* **48**:749-799.
- Loper, J. E., T. V. Suslow, and N. M. Schroth. 1984. Lognormal distribution of bacterial populations in the rhizosphere. *Phytopathology* **74**:1454-1460.
- Mosier, A. R., and W. J. Parton. 1985. Denitrification in a shortgrass prairie: a modeling approach, p. 441-452. In D. E. Caldwell et al. (ed.), *Planetary ecology*. Van Nostrand Reinhold Co., Inc., New York.
- Myrold, D. D. 1988. Denitrification in ryegrass and winter wheat cropping systems of western Oregon. *Soil Sci. Soc. Am. J.* **52**:412-416.
- Myrold, D. D., and J. M. Tiedje. 1985. Diffusional constraints on denitrification in soil. *Soil Sci. Soc. Am. J.* **49**:651-657.
- Parkin, T. B. 1985. Automated analysis of nitrous oxide. *Soil*

- Sci. Soc. Am. J. **49**:273–276.
20. **Parkin, T. B.** 1987. Soil microsites as a source of denitrification variability. *Soil Sci. Soc. Am. J.* **51**:1194–1199.
 21. **Parkin, T. B., A. J. Sexstone, H. F. Kaspar, and J. M. Tiedje.** 1984. A gas-flow soil core method to measure field denitrification rates. *Soil Biol. Biochem.* **16**:323–330.
 22. **Parkin, T. B., J. J. Meisinger, S. S. Chester, J. L. Starr, and J. A. Robinson.** 1988. Evaluation of statistical estimation methods for lognormally distributed variables. *Soil Sci. Soc. Am. J.* **52**:323–329.
 23. **Parkin, T. B., J. L. Starr, and J. M. Meisinger.** 1987. Influence of sample size on measurement of soil denitrification. *Soil Sci. Soc. Am. J.* **51**:1492–1501.
 24. **Rice, C. W., P. E. Sierzega, J. M. Tiedje, and L. W. Jacobs.** 1988. Simulated denitrification in the microenvironment of a biodegradable organic waste injected into soil. *Soil Sci. Soc. Am. J.* **52**:102–108.
 25. **Rice, C. W., and M. S. Smith.** 1982. Denitrification in no-till and plowed soils. *Soil Sci. Soc. Am. J.* **46**:1168–1173.
 26. **Robertson, G. P., and J. M. Tiedje.** 1984. Denitrification and nitrous oxide production in successional and old-growth Michigan forests. *Soil Sci. Soc. Am. J.* **48**:383–389.
 27. **Robinson, J. A.** 1985. Determining microbial kinetic parameters using nonlinear regression analysis: advantages and limitations in microbial ecology. *Adv. Microb. Ecol.* **8**:61–114.
 28. **Rolston, D. E., P. S. C. Rao, J. M. Davidson, and R. E. Jessup.** 1984. Simulation of denitrification losses of nitrate fertilizer applied to uncropped, cropped, and manure-amended field plots. *Soil Sci.* **137**:270–279.
 29. **Sexstone, A. J., N. P. Revsbech, T. B. Parkin, and J. M. Tiedje.** 1985. Direct measurement of oxygen profiles and denitrification rates in soil aggregates. *Soil Sci. Soc. Am. J.* **49**:645–651.
 30. **Smith, K. A.** 1980. A model of the extent of anaerobic zones in aggregated soils and its potential application to estimates of denitrification. *J. Soil Sci.* **31**:263–277.
 31. **Smith, M. S., and J. M. Tiedje.** 1979. Phases of denitrification following oxygen depletion in soil. *Soil Biol. Biochem.* **11**:261–267.
 32. **Tiedje, J. M.** 1982. Denitrification. *Agronomy* **9**:1011–1026.
 33. **Ugland, K. I., and J. S. Gray.** 1982. Lognormal distributions and the concept of community equilibrium. *Oikos* **39**:171–178.